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RESEARCH ARTICLE

Production of enantiomerically enriched chiral carbinols using whole-cell biocatalyst

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ABSTRACT

Biocatalytic asymmetric reduction of ketone is an efficient method for the production of chiral carbinols. The study indicates selective bioreduction of different ketones (**1–8**) to their respective (*R*)-alcohols (**1a–8a**) in low to high selectivity (0- >99%) with good yields (11–96%). In this work, whole-cell of *Lactobacillus kefiri* P2 catalysed enantioselective reduction of various prochiral ketones was investigated. (*R*)-4-Phenyl-2-butanol **2a**, which is used as a precursor to antihypertensive agents and spasmolytics (anti-epileptic agents), was obtained using *L kefiri* P2 in 99% conversion and 91% enantiomeric excess (ee). Moreover, bioreduction of 2-methyl-1-phenylpropan-1-one substrate **8**, containing a branched alkyl chain and difficult to asymmetric reduction with chemical catalysts as an enantioselective, to (*R*)-2-methyl-1-phenylpropan-1-ol **(8a)** in enantiomerically pure form was carried out in excellent yield (96%). The gram-scale production was carried out, and 9.70 g of (*R*)-2-methyl-1-phenylpropan-1-ol **(8a)** in enantiomerically pure form was obtained in 96% yield. Also especially, the yield and gram scale of (*R*)-2-methyl-1-phenylpropan-1-ol **(8a)** synthesised through catalytic asymmetric reduction using the biocatalyst was the highest report so far. The efficiency of *L kefiri* P2 for the conversion of the substrates and ee of products were markedly influenced by the steric factors of the substrates. This is a cheap, clean and eco-friendly process for production of chiral carbinols compared to chemical processes.

GRAPHICAL ABSTRACT

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Asymmetric reduction; whole-cell biocatalysts; *Lactobacillus kefiri*; chiral carbinol; biocatalytic transformation

Introduction

Chirality is important in living organisms due to the asymmetric nature of basic molecular building blocks, such as amino acids and sugars (Patel 2013). Enantiopure secondary alcohols possess significant importance as precursors for synthesis of complex active pharmaceutical intermediates, agrochemicals and other fine chemicals (Devendran and Yadav 2014; Gandomkar et al. 2015). In terms of biological effects, enantiomers of chiral compounds generally behave quite differently. Since most drugs act as single enantiomers, the synthesis of enantiomerically pure compounds is extremely important. The functional groups of chiral secondary alcohols can be easily converted to other functional groups without racemation (Hollmann et al. 2011). Several chemical processes are used to synthesise secondary alcohols such as chromatography separation, enantioselective crystallisation and asymmetric reduction of prochiral substrate using different chiral specific catalysts that are derived from transition metals such as Rh-complexes with different nitrogen containing compounds, chiral Lewis acid, metal-ligand complexes and oxazaborolidine (Touchard et al. 1999; He et al. 2014; Matsunami et al. 2018). However, there are several drawbacks associated with chemical processes such as requirement of expensive chiral ligands and hazardous metals, harsh conditions, low conversion and low enantioselectivity, and formation of byproducts. In addition to these chemical method, several procedures are available for obtaining optically pure alcohols like enzymatic resolution and catalytic reduction of ketones with biocatalysts (Şahin 2020a). Biocatalytic methods can offer highly selective, environmentally benign and energy effective solution for production of optically active compounds (Şahin and Dertli 2019; Şahin 2020b). Biocatalytic asymmetric reduction of prochiral ketones was reported to give >99% theoretical conversion with excellent ee as compared to other catalytic processes (Goldberg et al.

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Supplemental data for this article can be accessed here.

2007). Enantioselective bioreduction can be carried out by using either isolated enzymes or whole-cells. The bioreduction using microbial whole-cells as biocatalysts are advantageous than the purified enzymes, since the former contains multiple reductase enzymes and can accept a wide variety of unnatural substrates and synthesise necessary co-factors needed for biotransformation *in-situ* (Mandal et al. 2004). Enzymes usually require expensive co-factors for their activity, whereas whole-cell biocatalysts do not need them since they themselves have sufficient amount of co-factors. The use of whole-cell biocatalysts avoids a number of expensive methods required for isolation and purification of specific enzymes, and enzymes are also stable within the cells (Patel et al. 2004). The whole-cell biocatalysts during asymmetric reduction reaction is considered to be better than the purified enzymes (Rocha et al. 2009). Moreover, discovering new microbial strains is necessary to meet the demand of whole-cell biocatalyst in the industry.

There are a limited number of effectively useful *Lactobacillus kefiri* in the production of chiral carbinols which have been described in the literature. For instance, pure ADH enzyme isolated from *Lactobacillus kefiri* was used as biocatalyst for asymmetric reduction of aromatic ketones and different prochiral ketones to corresponding chiral secondary alcohols with high ee and conversion (Weckbecker and Hummel 2006). Ethyl (*S*)-4-chloro-3-hydroxybutanoate with high ee and yield was obtained by Amidjojo et al. using *Lactobacillus kefiri* as a whole-cell biocatalyst (Amidjojo and Weuster-Botz 2005). Asymmetric bioreduction of (2,5)-hexanedione to enantiomerically pure (5 *R*)-hydroxyhexane-2-one (ee $>$99%) with whole-cell immobilised *Lactobacillus kefiri* was carried out by Tan et al (Tan et al. 2006). Whole-cell *Lactobacillus kefiri* was not extensively studied for the asymmetric reduction of prochiral ketones to corresponding chiral secondary alcohols, which can be drug precursors. Considering the value of chiral carbinols, it will be increasingly important to identify biocatalysts that can be employed in the asymmetric bioreduction of different substrates. Thus in this work, we have investigated the reducing capacity of *L kefiri* P2 on various substrates.

Previous work shows that the *L kefiri* P2 is an important catalyst in the asymmetric reduction of various aromatic prochial ketones (Baydaş et al. 2020). Therefore, this study was aimed at increasing the substrate profile that *L kefiri* P2 can reduce, using previously achieved optimised conditions. Various prochiral substrates were reduced to corresponding secondary chiral alcohols with this *L kefiri* P2 using the optimisation conditions we obtained using the model substrate acetophenone in our previous study (Baydaş et al. 2020). Moreover, to the best of our knowledge, it is the first report showing that (*R*)-2-methyl-1-phenylpropan-1-ol **8a** is obtained as the enantiopure form using biocatalyst in the highest yield and maximum amount. In addition, the effects of different groups in substrates on selectivity and transformation were evaluated. The current study offers a practical method for preparation of chiral secondary alcohols.

Materials and methods

General

The substrates, solvents and the growth medium of bacteria (MRS) were purchased from Fluka and Sigma-Aldrich. Purification of **1a–8a** were carried out by column chromatography and the alcohols were obtained using ethyl acetate/hexane: (15:85, v/v) solvent mixture. Progress of reaction was checked by Thin layer chromatography (TLC), using ethyl acetate: hexane (10:90, v/v) as the mobile phase. All the racemic alcohols (**1a–8a**) were prepared by reducing the corresponding ketones with sodium borohydride in methanol. HPLC analysis was performed on an Agilent chromatograph (Model no: 1260). The product characterisation was determined by Bruker NMR spectroscopy (Bruker Ltd., Germany). Bellingham + Stanley (ADP220, 589 nm) spectropolarimeter was used for the optical rotation of the product. Conversion was determined by the HPLC using comparison of the ketone peak with the alcohol peak after HPLC analysis of the crude product.

Culture medium and bacterial strain

L kefiri P2 strain used in this study was previously isolated from kefir and this strain was grown in MRS broth as described previously (Baydaş et al. 2020).

General procedure for asymmetric bioreduction

L kefiri P2 was added from its glycerol stock by inoculation to 10 mL MRS medium ($MgSO_4.7H_2O$ 11.5% (w/v), K_2HPO_4 2 g/L, pepton [Oxoid] 10 g/L, yeast extract 2% glucose, [Difco] 5 g/L, $C_2H_3NaO2.3H_2O$ 5 g/L, salt solution [MgSO4.7H2O 11.5% (w/v), triamonium citrate 2 g/L], Tween 80 1 mL/L) followed by 48 h growth at 37 °C. From this mixture, overnight grown bacterial cells were inoculated to 100 mL MRS mixture at 10% concentration and then pH was adjusted to 4.5

using1M HCl. After adjusting the pH, the mixture was incubated again at 25 °C, 150 rpm in an incubator-shaker for 2 h. Then, 1 mmol of substrates (**1–8**) were added to the reaction medium and the reactions were incubated again at 25 °C, 150 rpm in an incubator-shaker for 64 h. The supernatant was extracted with CH_2Cl_2 and saturated with NaCl, then the product was obtained and tested as described previously (Baydaş et al. 2020). HPLC analysis was applied for the determination of the ee of the products and the yields were determined following the purification process in column chromatography.

Gram scale synthesis of (R)-2-methyl-1-phenylpropan-1-ol (8a)

L kefiri P2 was added from its glycerol stock by inoculation to 10 mL MRS medium followed by 48 h growth at 37 °C. From this mixture, overnight grown bacterial cells were inoculated to 2 L MRS medium at 10% concentration and incubation for 2 h under optimisation conditions. Then the pH of the mixture was adjusted to 4.5 by 1 M HCl. Following the 2 h of incubation, substrate **8** (10 g) was added to the mixture and incubated on an incubator-shaker at 25 °C, 150 rpm for 64 h. The product was obtained from the culture supernatant purified and characterised as described above.

(*R*)-2-bromo-1-(naphthalen-2-yl)ethanol (1a) (Kang et al. 2017)**:** White solid, M.p.: 66-68 °C, ^{1}H-NMR (400 MHz, $CDCl_3$) $\delta = 7.88–7.79$ (m, 3H), 7.52–7.43 (m, 4H), 5.04 (dd, $J = 4.1$, 2.6 Hz, 1H), 3.72 (dd, $J = 5.4$, 4.1 Hz, 1H), 3.60 (dd, $J = 5.4$, 2.6 Hz, 1H), 2.75 (d, $J = 2.6$ Hz, 1H (OH)); ^{13}C-NMR (100 MHz, $CDCl_3$) $\delta = 135.0$, 133.3, 128.4, 127.9, 127.8, 126.4, 126.3, 126.1, 125.2, 122.6, 72.8, 39.9; $[\alpha]_D^{25} = +15.4$ (c 1.1, $CHCl_3$), 32% ee; Lit. $[\alpha]_D^{25} = +47.1$ (c 1.1, $CHCl_3$, 98% ee for *R* enantiomer) (Kang et al. 2017); HPLC conditions: Chiralcel AS-H column, 254 nm, flow rate: 1.0 mL/min, *i*-PrOH/*n*-hexane 5:95, t_R (*R*) 18.7, (*S*) 16.2 min (Figure S10). HPLC analysis condition of ketone **1** is the same as alcohol **(*R*)-1a** and retention time of substrate was determined as 20.8 min (Supporting information).

(*R*)-4-phenylbutan-2-ol (2a) (Salvi and Chattopadhyay 2016): Colourless oil, ^{1}H-NMR (400 MHz, $CDCl_3$) $\delta = 7.29–7.21$ (m, 5H), 3.83 (m, 1H), 2.75–2.67 (m, 2H), 1.77–1.64 (M, 2H), 1.42 (s, 1H), 1.23 (d, $J = 6.0$ Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$)) $\delta = 142.0$, 128.4, 125.8, 67.5, 40.8, 32.1, 23.6; $[\alpha]_D^{25} = -15.5$ (c 0.57, $CHCl_3$), 91% ee; Lit. $[\alpha]_D^{25} = +16.5$ (c 0.57, $CHCl_3$, 96.4% ee for *S* enantiomer) (Salvi and Chattopadhyay 2016); HPLC conditions: Chiralcel OD column, 254 nm, and flow rate: 1.0 mL/min, *i*-PrOH/*n*-hexane 5:95, t_R (*R*) 14.5, (*S*) 21.8 min. HPLC analysis condition of ketone **2** is the same as alcohol **(*R*)-2a** and retention time of substrate was determined as 9.3 min.

(*R*)-1-(naphthalen-2-yl)ethanol (3a) (Wu et al. 2016): Colourless oil, ^{1}H-NMR (400 MHz, $CDCl_3$) $\delta = 7.85$ (m, 4H), 7.52 (m, 3H), 5.07 (q, $J = 6.5$ Hz, 1H), 2.21 (bs, OH), 1.60 (d, $J = 6.4$ Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$))) $\delta = 143.2$, 133.3, 132.9, 128.3, 128.0, 127.7, 126.2, 125.8, 123.9, 123.8, 70.5, 25.2; $[\alpha]_D^{25} = +30.6$ (c 0.50, CH_2Cl_2), 82% ee; Lit. $[\alpha]_D^{25} = +37.0$ (c 0.50, CH_2Cl_2, 99% ee for *R* enantiomer) (Wu et al. 2016); HPLC conditions: Chiralcel AS-H column, 230 nm, flow rate: 1.0 mL/min, *i*-PrOH/*n*-hexane 3:97, t_R (*R*) 19.7, (*S*) 23.4 min. HPLC analysis condition of ketone **3** is the same as alcohol **(*R*)-3a** and retention time of substrate was determined as 15.8 min.

(*R*)-cyclohexyl(phenyl)methanol (4a) (Carlos et al. 2015): White solid, M.p.: 64–66 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) $\delta = 7.36–7.28$ (m, 5H), 4.39 (d, $J = 7.2$ Hz, 1H), 2.01–1.61 (m, 6H), 1.42–0.94 (m, 6H); ^{13}C-NMR (100 MHz, $CDCl_3$) $\delta = 143.6$, 128.2, 127.4, 126.6, 79.4, 45.0, 29.3, 28.8, 26.4, 26.1, 26.0; $[\alpha]_D^{25} = -11$ (c 0.50, CH_2Cl_2), 28% ee; Lit. $[\alpha]_D^{25} = -28$ (c 0.50, CH_2Cl_2, %71 ee for *R* enantiomer) (Carlos et al. 2015); HPLC conditions: Chiralcel OD-H column, 254 nm, flow rate: 1.0 mL/min, *i*-PrOH/*n*-hexane 1:99, t_R (*R*) 25.5, (*S*) 19.9 min. HPLC analysis condition of ketone **4** is the same as alcohol **(*R*)-4a** and retention time of substrate was determined as 5.9 min.

(*R*)-2,3-dihydro-1H-inden-1-ol (5a) (Guo et al. 2015): White solid, M.p.: 68–69 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) $\delta = 7.46–7.37$ (m, 1H), 7.30–7.20 (m, 3H), 5.29–5.20 (m, 1H),3.06–2.99 (m, 1H), 2.89–2.74 (m, 1H), 2.56–2.38 (m, 1H), 2.05–1.82 (m, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) $\delta = 145.0$, 143.3, 128.3, 126.7, 124.9, 124.2, 59.5, 35.9, 29.7; $[\alpha]_D^{25} = -16.1$ (c 0.88, $CHCl_3$), 52% ee; Lit. $[\alpha]_D^{25} = -30.7$ (c 0.88, $CHCl_3$, %98.9 ee for *R* enantiomer) (Guo et al. 2015); HPLC conditions: Chiralcel AS-H column, 220 nm, flow rate: 0.5 mL/min, *i*-PrOH/*n*-hexane 1:99, t_R (*R*) 51.3, (*S*) 36.3 min. HPLC analysis condition of ketone **5** is the same as alcohol **(*R*)-5a** and retention time of substrate was determined as 34.6 min.

(4-nitrophenyl)(phenyl)methanol (6a) (Karthikeyan et al. 2010): White solid, M.p.: 76–78 °C; ^{1}H-NMR (400 MHz, $CDCl_3$) $\delta = 8.18$ (dd, $J = 8.8$ Hz, 2H), 7.56 (d, $J = 8.8$ Hz, 2H), 7.29–7.37 (m, 5H), 5.90 (s, 1H), 2.50 (s, 1H); ^{13}C-NMR (100 MHz, $CDCl_3$) $\delta = 150.7$, 142.7, 128.9, 128.8, 128.4, 127.0, 126.7, 123.7, 75.5;

$[\alpha]_D^{25} = 0$ (c 0.30, $CHCl_3$), 0% ee; Lit. $[\alpha]_D^{25} = +57.7$ (c 0.30, $CHCl_3$, %93 ee for *S* enantiomer) (Karthikeyan et al. 2010); HPLC conditions: Chiralcel OD-H column, 250 nm, flow rate: 0.9 mL/min, *i*-PrOH/*n*-hexane 20:80, t_R (*R*) 11.1, (*S*) 12.2 min. HPLC analysis condition of ketone **6** is the same as alcohol **6a** and retention time of substrate was determined as 10.6 min.

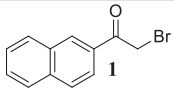
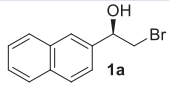
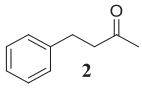
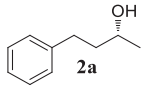
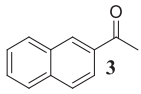
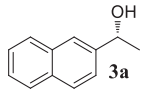
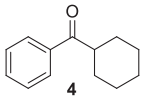
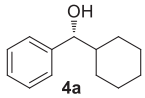
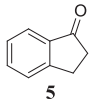
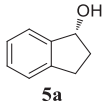
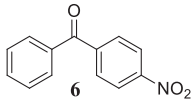
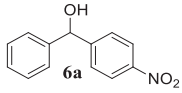
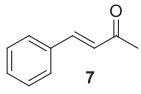
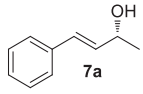
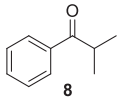
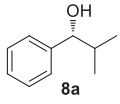
(*R*)-4-phenylbut-3-en-2-ol (7a) (Chen et al. 2017): Yellow oil, ^{1}H-NMR (400 MHz, $CDCl_3$) $\delta = 7.39–7.27$ (m, 5H), 6.57 (d, $J = 15.8$ Hz, 1H), 6.28 (d, $J = 15.9$ Hz, 1H), 4.48 (m, 1H), 2.6 (bs, 1H (OH)), 1.39 (d, $J = 6.4$ Hz, 3H); ^{13}C-NMR (100 MHz, $CDCl_3$) $\delta = 136.8$, 133.7, 129.2, 128.6, 127.6, 126.5, 68.8, 23.4; $[\alpha]_D^{25} = +7.2$ (c 1.0, $CHCl_3$), 22% ee; Lit. $[\alpha]_D^{25} = -32.6$ (c 1.0, $CHCl_3$) for 99% ee for *S* enantiomer) (Chen et al. 2017); HPLC conditions: Chiralcel OD-H column, 254 nm, flow rate: 1.0 mL/min, *i*-PrOH/*n*-hexane 10:90, t_R (*R*) 9.2, (*S*) 14.1 min. HPLC analysis condition of ketone **7** is the same as alcohol **(*R*)-7a** and retention time of substrate was determined as 8.3 min.

(*R*)-2-methyl-1-phenylpropan-1-ol (8a) (Yu et al. 2017): Colourless oil, purified yield 96%, ^{1}H NMR (400 MHz, $CDCl_3$) $\delta = 7.36–7.25$ (m, 5H), 4.36 (dd, $J = 6.8$, 3.1 Hz, 1H), 2.00–1.90 (m, 1H), 1.87 (d, $J = 3.1$ Hz, 1H), 1.62 (bs, 1H, (OH)), 1.00 (d, $J = 6.8$ Hz, 3H), 0.80 (d, $J = 6.8$ Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) $\delta = 143.6$, 128.2, 127.4, 126.5, 80.0, 35.3, 19.0, 18.2; $[\alpha]_D^{25} = 41.6$ (c = 1, $CHCl_3$) >99% ee; Lit. (*R*)-2-methyl-1-phenylpropan-1-ol, $[\alpha]_D^{20} = 41.6$ (c = 1, $CHCl_3$, for 99%ee) (Yu et al. 2017); HPLC conditions: Chiralcel OD-H column, 220 nm, flow rate: 1 mL/min, *n*-hexane/*i*-PrOH 98:2, t_R (*R*) 13.8 min. HPLC condition of substrate **8** is the same as alcohol **(*R*)-8a** and retention time of substrate was determined as 5.0 min.

Results and discussion

In this study, the biocatalytic reactions were carried out using 1 mmol substrates (**1–8**) under optimised conditions which is obtained in the previous study (Table 1). In our previous study, asymmetric reduction reaction conditions were optimised using the model substrate acetophenone with *L kefiri* P2 and optimisation conditions were obtained as pH 4.5, time 64 h, temperature 25 °C, agitation speed 150 rpm (Baydaş et al. 2020). Enantiomerically enriched chiral 2-haloethanols are applied extensively, as these building blocks can be readily converted into various β-adrenergic receptor agonists and blockers (Ye et al. 2015). Nifenalol, pronethalol, metaproterenol, and fenoterol are among important medicines prepared from aryl-substituted 2-haloethanols (Kapoor et al. 2005; Jozwiak et al. 2007; Wei et al. 2011; Bosak et al. 2012). Enantiopure alcohol **1a**, which is 2-haloethanols, was obtained by kinetic resolution of the corresponding racemic alcohol **1a** (Hiratake et al. 1988) and asymmetric transfer hydrogenation of 2-halo ketones **1** with a chemical catalyst (Zhou et al. 2016). The *L kefiri* P2 mediated bioreduction of substrate **1** was reduced to the corresponding (*R*)-carbinol **1a** in 65% yield and with 32% ee (Table 1, entry 1). Bioreductive products of ketones type $Ar(CH_2)nCOR$ ($n > 0$, R = alkyl) are synthetically important because the aryl moiety of the resultant chiral alcohols can be oxidatively cleaved to furnish chiral hydroxyl acids (Carlsen et al. 1981), which are present in various bioactive natural products (White and Amedio 1989). 4-Phenyl-2-butanol is also important to the pharmaceutical industry because it is used as a precursor to antihypertensive agents and spasmolytics (anti-epileptic agents) (Liese et al. 2000). The reduction of 2 had been previously reported using different microbial strain such as *Geotrichum candidum* (Matsuda et al. 2006), recombinant diketoreductase (Wu et al. 2009), and purified ketoreductases (KREDs) (Cicco et al. 2018) with high selectivity. The *L kefiri* P2 mediated bioreduction of substrate **2**, with two-atom spacer between the phenyl and ketone groups, was reduced to the (*R*)-4-phenylbutan-2-ol **2a** in 99% yield and with 91% ee (Table 1, entry 2). The asymmetric synthesis of aryl methyl carbinols, a highly versatile and fascinating group of chiral building blocks for the synthesis of molecules with desirable biological activities was carried out extensively (Patel 2002; Panke and Wubbolts 2005). Tozlu et al. reported that enantiomerically enriched (*S*)-1-(naphthalene-2-yl)ethanol **3a** using *Weissella paramesenteroides* was obtained with 72% ee and 77% yield (Tozlu et al. 2019). Besides, the enantioselective reduction of the prochiral aryl methyl ketone derivative **3** was performed with high selectivity, using different biocatalysts by many research groups (Maczka and Mironowicz 2002; Yadav et al. 2002; Homann et al. 2004; Muthineni et al. 2016). Here, (*R*)-1-(naphthalen-2-yl)ethanol **3a** was obtained with *L kefiri* P2 in 82% ee and 68% yield (Table 1, entry 3). There are numerous studies involving enantioselective reduction of cyclohexyl (phenyl) methanone **4** with chemical catalysts (Hayes et al. 2005; Bigler et al. 2015; Slagbrand et al. 2015), but there are limited studies involving the bio-reduction of this compound (Şahin et al. 2019). Ema *et al.* reported that (*S*)-**4a** was obtained with 59% ee by the lipase enzyme (Ema et al. 2009). Chartrain *et al.* demonstrated that cyclohexyl(phenyl)methanone **4** is reduced to (*R*)-**4a** by 75% ee and 55% conversion

Table 1. Asymmetric bioreduction of various prochiral ketones (**1–8**) using *L kefiri* P2.

Entry	Substrate	Product	ee (%)[a] (*R*)[b]	Conversion(%)[c]	Yield (%)[d]
1			32	68	65
2			91	99	94
3			82	71	68
4			28	14	11
5			52	99	95
6			Racemic	14	12
7			22	94	91
8			>99	>99	96

[a]Determined by HPLC using [a]Chiral column, [b]Determination of absolute configuration was carried out by comparison of the sign of optical rotation relative to the values in the literature, [c]The conversions were determined by chiral HPLC, [d]isolated yield.

with yeast *Candida magnolia* (Chartrain et al. 1997). In this study, cyclohexyl(phenyl)methanone **4** was reduced to (*R*)-cyclohexyl(phenyl)methanol **4a** with 28% ee and 14% conversion (Table 1, entry 4). The analog of indanol-(*1S,2R*)-1-amino-2-indanol is used as a precursor in the production of Indinavir (Crixivan®), which acts as the HIV protease inhibitor in antiretroviral therapy (Mączka et al. 2019). Numerous biocatalysts were used for the stereoselective reduction of the prochiral 1-indanone **5** having a cyclic structure, however 1-indanol **5a** was obtained at low yield with high selectivity (Caron et al. 2005; Yadav et al. 2009; Nagai et al. 2018). It has also been reported in the literature that fused cyclic ketones such as substrate **5** are difficult to reduce with biocatalysts (Sun et al. 2016). Here, substrate **5** was reduced to (*R*)-2,3-dihydro-1H-inden-1-ol **5a** in 99% conversion and with 52% ee (Table 1, entry 5). Diaryl carbinols are important synthetic building blocks in the manufacturing of pharmaceuticals, such as (*R*)-orphenadrine, (*R*)-neobenodine, (*S*)-cetirizine, carbinoxamine, and *L*-cloperastine, which possesses antitussive and antiedemic activity, relaxes the bronchial musculature (Schmidt et al. 2006; Magnus et al. 2007; Wujkowska et al. 2016). The asymmetric hydrogen transfer to diaryl ketones were performed to form diarylmethanols with high selectivity; however, these reactions have serious drawbacks for large-scale production in their potential application (Touge et al. 2016). Biocatalysts have been demonstrated to be stereoselective in the reduction of some diaryl ketones that are difficult to reduce with chemical catalysts. Truppo et al. have shown that many diaryl ketones consisting of mono and disubstituted benzophenones have been successfully reduced to their respective diarylmethanols with moderate to excellent selectivity and small scale by using commercially available ketoreductase enzyme (Truppo et al. 2007). Sahin studied the reduction of a series of diaryl aromatic ketones catalysed by whole-cell of *C. zeylanoides* P1, wherein he has obtained excellent ee and yield (Şahin 2020c). When the asymmetric reduction of biaryl ketone **6** with *L kefiri* P2, **6a** was obtained with racemate and low conversion (Table 1, entry 6). This low conversion and selectivity can be explained by

the sterically large substrate. α, β-Unsaturated seconder alcohol **7a** is used as a precursor in the total synthesis of Chatenaytrienin-2 that is a group of fatty acid type natural products that shown to possess interesting and important bioactivities, significantly the antitumor effects, and growth inhibitory action against multidrug-resistant cancer cells (Kunkalkar and Fernandes 2019). Chemical chiral ligands are generally used for preparing chiral allylic alcohols through asymmetric 1,2-reduction of enones. As an alternative to these chemical methods, effective enzymatic methods have been developed for the preparation of this chiral alcohols (Nishihara et al. 2017; Kadotani et al. 2019). Among them, enzymatic kinetic resolution of racemic alcohols and asymmetric reduction of enzyme-catalysed prochiral ketones are the main approaches. The theoretical maximum yield of the enzymatic kinetic resolution is 50%, which greatly limits the application of this method. Cai et al. described that bioreduction of substrate **7** using *Perakine Reductase* as biocatalyst was carried out to give (*S*)-carbinol **7a** in >99% ee and 60% yield (Cai et al. 2019). The *L kefiri* P2 mediated bioreduction of substrate **7** was reduced to the corresponding (*R*)-carbinol **7a** in 91% yield and with 22% ee (Table 1, entry 7). (*S*) or (*R*)-2-methyl-1-phenylpropan-1-ol **(8)** was synthesised in the high ee using different chemical process such as asymmetric reduction of ketone with chiral ligand and asymmetric alkylation of the corresponding aldehyde (Ling et al. 2018; Sato et al. 2000). However, there is only one study in which 2-methyl-1-phenylpropan-1-one **(8a)** was obtained in enantiomerically pure form reduced from corresponding ketone by using biocatalyst in the literature (Şahin 2020d). The *L kefiri* P2 mediated bioreduction of substrate **8** was reduced to the corresponding (*R*)-**8a** in 96% yield and with >99% ee (Table 1, entry 8). Since **8** afforded excellent enantioselectivity and conversion for the reduction on a small scale, we decided to conduct the transformation of **8** to **(*R*)-8a** on a large scale to demonstrate the viability of the present system as industrially feasible (Figure 1). The best ee (>99%) of product and complete conversion were achieved with 67.4 mmol/2L concentration of substrate **8** (Figure 1). Under optimised conditions, substrate **8** (76.4 mmol, 10.0 g) was converted to (*R*)-2-methyl-1-phenylpropan-1-ol **8a** (9.70 g) using *L kefiri* P2 in 96% yield with >99% ee and conversion. Compared with the past report, which used biocatalyst, 2-methyl-1-phenylpropan-1-one **8** was bioreduced to (*R*)-2-methyl-1-phenylpropan-1-ol **8a** in higher yield and gram scale. These results show that *L kefiri* P2 is an important biocatalyst for the production

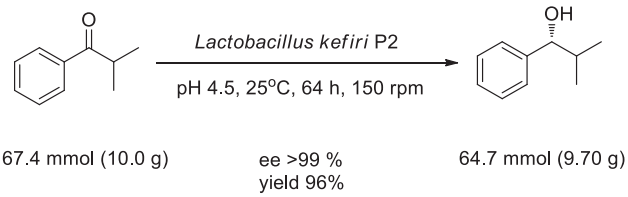


Figure 1. Gram scale synthesis of (*R*)-2-methyl-1-phenylpropan-1-ol **(8a)**.

of (*R*)-2-methyl-1-phenylpropan-1-ol **8a** and this biocatalyst can be used industrially. Low conversion was observed with the bulkier substrates **4** and **6** (Table 1, entry 4, 6). The reason for the low conversion of substrates **4** and **6** may be due to the low solubility of these substrates in water. However, it was observed that there were no changes in the conversion and ee in using organic solvents such as 5% ethyl alcohol, isopropyl alcohol and DMSO under optimised conditions. These results shows that solubility is not the cause for low conversion and ee for these substrates. The low conversion rate for these substrates can be explained by the inability of bulky groups to interact with the enzyme active site. When substrate **2** and **7** are compared, it is seen that the conversion is high for both substrates. However, the selectivity obtained in substrate **2** is quite high compared to **7** (Table 1, entry 2, 7). This shows that substrate **2** interacts better with the enzyme active site. When the substrates **1** and **3** are compared, **1a** was obtained with lower conversion and ee. This observation can be explained in terms of steric hindrance of the substituent. Alsafadi and coworkers has previously reported a similar trend (Alsafadi et al. 2017). These results show that the reduction and selectivity efficiency of *L kefiri* P2 varies depending on the steric activity of the substrates.

Conclusion

In conclusion, we have investigated the use of *L kefiri* P2 for the production of chiral carbinols with high ee and yields. We have shown that *L kefiri* P2 isolated from kefir effectively catalyses the enantioselective reduction of a various ketones (**1–8**) to give the (*R*)-form of carbinols (**1a–8a**) with up to 0–99% ee. (*R*)-2-methyl-1-phenylpropan-1-ol **8a**, which is difficult to reduce with chemical catalysts as an enantioselective in the literature, was synthesised in gram scale, excellent yield and enantiomerically pure form using *L kefiri* P2. These results showed that current process has significant potential for the green synthesis of (*R*)-2-methyl-1-phenylpropan-1-ol **8a** at an industrial scale. This efficient and eco-friendly method was found to be a versatile and promising tool for the clean

production of chiral secondary alcohols. The results are promising the suitability of *L kefiri* P2 as a valuable biocatalyst for the synthesis of chiral carbinols of pharmaceutical interest. At the same time, asymmetric reduction studies of substrates that may be important for the pharmaceutical industry with this biocatalyst continue in our laboratory.

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Disclosure statement

No potential conflict of interest was reported by the author(s).

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